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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 335/73 | A2

(11) International Publication Number: **WO 99/64869**

(43) International Publication Date: 16 December 1999 (16.12.99)

(21) International Application Number: PCT/GB99/01823

(22) International Filing Date: 9 June 1999 (09.06.99)

(30) Priority Data:
9812290.6 9 June 1998 (09.06.98) GB

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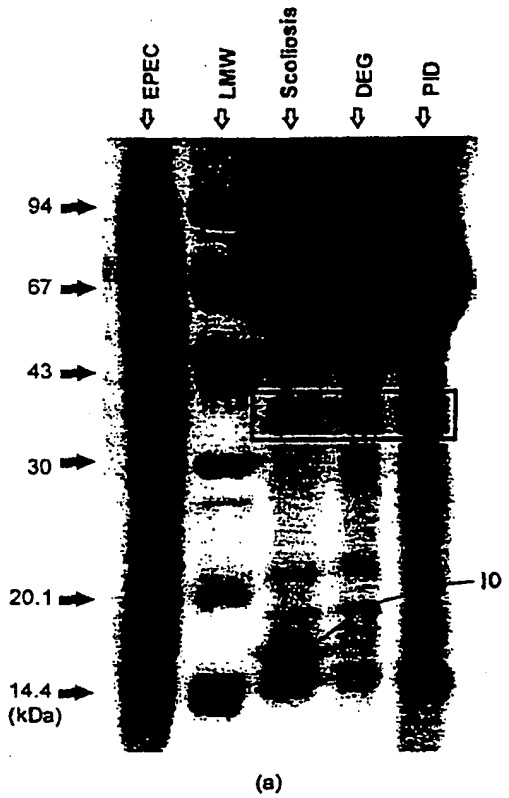
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: BACK PAIN DIAGNOSIS AND APPARATUS FOR USE THEREIN

(57) Abstract

The intervertebral disc is implicated as a source of symptoms of low back pain and sciatica. Degenerative disc disease and inflammatory processes may result in low back pain. The invention identifies the involvement of lysozyme C and tyrosine phosphorylation in low back pain, sciatica and other joint diseases and provides methods and apparatus for diagnosis and uses of these substances, and processes and substances influencing these processes in the treatment and diagnosis of such diseases and conditions.



(a)

BACK PAIN DIAGNOSIS AND APPARATUS FOR USE THEREIN

The present invention relates to the diagnosis and therapy of back pain, and apparatus for use therein.

Back pain is, after the everyday cold, the most common health problem in Britain and America. Eighty percent of the population will experience the symptoms of back pain. It is believed that the annual cost of treating back pain in the UK is approximately £5 billion.

The intervertebral disc is implicated as a source of symptoms in the majority of patients with low back pain and sciatica, particularly in relation to sciatica secondary to disc prolapse. Degenerative disc disease is characterised by the gradual loss of water and proteoglycan from the nucleus pulposus which in part leads to biochemical changes within the disc resulting in height loss and disc prolapse.

It has been implied that inflammatory processes may be activated resulting in low back pain. In addition there may also be a series of related biochemical processes that may be activated resulting in disc degeneration and the generation of inflammatory pain.

In this specification the term "lysozyme C" is to be understood as including any lysozyme C like proteins which have a similar N-terminal amino acid sequence as found in lysozyme C.

According to the present invention there is provided a method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease, or a predisposition thereto, said method comprising detecting the presence of lysozyme C in tissue extracted from intervertebral discs or fluid extracted from large joints.

Preferably the amount of lysozyme C is detected.

Preferably the method involves analysis of the degree of change in the magnetic resonance properties of the homologue, which may provide for determination of the degree of disease progression.

Further according to the present invention there is provided apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a support on which an unconjugated monoclonal antibody to lysozyme C is immobilised whereby an indication is given when the support comes into contact with lysozyme C.

The support preferably comprises a test strip or a nitro-cellulose/nylon sandwich membrane. The test strip may comprise opaque polystyrene or another support matrix which is able to bind antibodies. The support is preferably pre-blocked with blocking agents when the unconjugated antibody has been immobilised thereon.

The apparatus is preferably utilised in an enzyme linked immunosorbent assay using an enzyme conjugated monoclonal antibody to lysozyme C. Preferably the enzyme is alkaline phosphatase, horse radish peroxidase or biotin. The substrate for the enzyme preferably provides a colour change as an indication of the presence of lysozyme C.

The invention may further comprise a method of treatment of low back pain and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of lysozyme C.

The lysozyme C may be administered by injection into a vertebral disc or joint. The lysozyme C may be administered in combination with a growth factor and/or a phosphatase inhibitor. The method may be used to treat joint disease.

Still further according to the present invention there is provided a

phosphorylated annexin.

The support preferably comprises a test strip or a nitro-cellulose/nylon sandwich membrane. The test strip may comprise opaque polystyrene or another support matrix which is able to bind antibodies. The support is preferably pre-blocked with blocking agents when the unconjugated antibody has been immobilised thereon.

The apparatus is preferably utilised in an enzyme linked immunosorbent assay using an enzyme conjugated monoclonal antibody to tyrosine phosphate. Preferably the enzyme is alkaline phosphatase, horse radish peroxidase or biotin. The substrate for the enzyme preferably provides a colour change in the presence of tyrosine phosphorylated annexin.

The invention further provides apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a support on which an unconjugated monoclonal antibody to annexin and an unconjugated antibody to lysozyme C are immobilised whereby an indication is given when the support comes into contact with tyrosine phosphorylated annexin and lysozyme C.

The two antibodies are preferably immobilised on separate areas of the support. The support preferably comprises a test strip or a nitro-cellulose/ nylon sandwich membrane. The test strip may comprise opaque polystyrene or another support matrix which is able to bind antibodies. The support is preferably pre-blocked with blocking agents when the unconjugated antibodies have been immobilised thereon.

The apparatus is preferably utilised in an enzyme linked immunosorbent assay using enzyme conjugated monoclonal antibodies to lysozyme C and tyrosine phosphate. Preferably the enzyme is alkaline phosphatase, horse radish peroxidase or biotin. The substrate for the enzyme preferably provides a

The invention also provides for a method of treatment of low back paid and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of tyrosine phosphorylation annexin.

The method may be used to treat joint disease.

The invention may further provide a method of diagnosing low back paid and sciatica, or a predisposition thereto, the method comprising analysing the presence of tyrosine phosphorylation or phosphatase and of lysozyme C in tissue extracted from the invertebral discs or fluid extracted from large joints. The method may also be used to diagnose joint disease.

The invention further provides for the use of lysozyme C and a phosphatase inhibitor in the manufacture of a medicament for the treatment of low back pain and sciatica. The invention may further be used to treat joint disease.

Further according to the present invention there is provided a method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said method comprising detecting the presence of mutations in the lysozyme C gene in blood samples taken from the body.

The method comprises using a set primer for these mutations in conjunction with a standard primer with no mutation to amplify the gene fragments by the Polymerase Chain Reaction (PCR) if the mutation is present.

Alternatively the method may comprise synthesising primers with the non-mutant sequences in the 5' end for all mutation regions of the lysozyme C gene. Preferably the set of primers is used as a mixture thereby enabling DNA fragments to be amplified using PCR on the blood DNA to produce a DNA profile. Preferably the absence of certain bands from the profile is indicative of disease.

The invention still further provides use of corticosteroids as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

Preferably the corticosteroid is injected in combination with intradiscal or intrajoint injection of lysozyme C or with lysozyme C and phosphatase inhibitor.

The invention may further provide for the use of corticosteroids for use in the manufacture of a medicament for the treatment of back pain and sciatica.

The invention yet further provides use of growth factors as therapeutic agents in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

The growth factors are preferably epidermal growth factor, platelet derived growth factor or hormones such as insulin, or any agonist which will restore the tyrosine phosphorylation of annexins.

The invention further provides the use of tyrosine phosphorylation annexins in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease.

Preferably the growth factors may be injected in combination with any or all of lysozyme C, phosphatase inhibitor and corticosteroids.

The invention further provides for the use of tyrosine phosphoylated annexins in the manufacture of a medicament for the treatment of low back pain and sciatica.

The invention further provides for the use of growth factors in the manufacture of a medicament for the treatment of back pain and sciatica.

'signal transduction' processes causes a shift in the equilibrium of enzymes, themselves proteins, and this is translated into alterations in the protein concentrations, and the result of this is seen as differences in the protein profiles within the cell.

The samples assayed were the nucleus pulposus (NP) which were carefully excised from the intervertebral disc. Patient groups studied were split into four groups:

'DISEASED PATIENTS':

1) PID (prolapsed intervertebral disc) = sciatica cases undergoing disc resection
2) DEG (degenerate disc) = mainly back pain cases undergoing anterior spinal fusion

'NORMAL PATIENTS':

3) Scoliosis (normal) = scoliosis cases undergoing disc resection and correction

4) Donor (normal) = disc removed from patients undergoing organ donation, with no past history of back pain.

RESULTS:

a) Tyrosine-Phosphorylated protein(s):

NP samples were processed and probed with anti-phosphotyrosine antibodies for the presence of Tyr-P proteins (12,14) using the Western hybridisation method. The four patient groups, detailed as above, were investigated. Results showed a significantly higher level of phosphorylation of tyrosine residues from NP proteins in the normal groups (group 3 + 4) as compared to the diseased groups (group 1 + 2).

annexin for use in the manufacture of a medicament for the treatment of low back pain and sciatica and the use of phosphatase inhibitors in the treatment, diagnosis and manufacture of medicaments for such treatment.

b) Lysozyme C

NP samples from the four groups were prepared and denatured proteins separated by SDS-agarose gel electrophoresis. The proteins were then visualised with Coomassie Blue staining. The major protein difference was a protein 10 with a molecular weight of approximately 15 kDa identified by N-terminal amino acid sequencing as lysozyme C as shown in Fig. 1. Results showed a significantly higher level of lysozyme C within the NP of the normal groups (group 3 + 4) as compared to the diseased groups (group 1 + 2). See Table 2 below.

Table 2. Comparison of the levels of lysozyme C in the four study groups.

Levels of Lysozyme C

Group (N=)	High	Medium	Low	Absent
1-PID (17)[+]	0	0	2	15
2-DEG (16)[+]	0	2	7	7
3-Scoliosis (16)[+]	13	3	0	0
4-Donor (4)[++]	3	0	0	0

[+] = highly significant ($P<0.001$)

[++] = number too small for statistical analysis

Lysozyme C is a secretory protein normally found in tears, saliva, breast milk, etc. and functions specifically in digestion of bacterial cell wall proteins. The significance of its presence within the intervertebral disc is not fully understood. The question raised is whether this enzyme has secondary

In the light of the above results, the following diagnostic methods and test kits, and therapies were produced.

(1) LYSOZYME C DETECTION

Reagents:

a) 2 monoclonal antibodies to spinal disc lysozyme C, both recognise denatured and native protein. But both recognise different epitopes of lysozyme C. One remains unconjugated (UC) and is used in the test strip only, the other is conjugated to alkaline phosphatase (AP) and is used for detection in all tests.

b) Extraction buffer: 10 mM Tris-Hcl pH 7.0
1 mM EDTA
1-3% non ionic detergent (e.g. TritonX100) for native
OR if denatured
Sodium dodecyl sulphate (SDS).

c) blocking buffer: 3% serum albumin
0.5% Tween
in 10 mM Tris pH 7.0

d) fixing solution: 1% trichloro-acetic acid (TCA).

e) incubation buffer: 10 mM Tris-Hcl pH 7.0

f) AP substrate: BCIP (5-bromo 4-chloro 3-indoylphosphate) and
NBT (nitro blue tetrazolium).

Dissolved in 10 mM Tris-Hcl pH 8.5

(A) For basic lab based Western blot test:

sandwich or another support matrix which is able to bind antibodies. The test strip is pre-blocked once antibody has been immobilised. The tissue from the needle biopsies is chopped up and removed to 2X volumes of extraction buffer containing non-ionic detergent to keep protein native. The tissue is incubated in extraction buffer with occasional mixing for 15 minutes heated at 65°C (heating may not be needed). After this time 10-20 µl aliquots of extract is placed on the area of the test strip where the UC monoclonal antibody is immobilised, and left for 10-20 minutes for the lysozyme C to bind. The strip is then washed in excess 10 mM Tris pH 7.0 and 10-20 µl of the AP-conjugated monoclonal antibody placed on the same position of the strip. This is left for approx. 20 minutes and then washed in excess Tris pH 7.0 and then immersed in the substrate solution for 10 minutes. The presence of lysozyme C will be shown as a dark spot on the test strip, indicating a healthy disc.

D) Colorimetric assay:

Lysozyme is a β-N-acetylmuramidase which hydrolyses the β-1,4-glycosidic bond between N-acetylmuramic acid (MurNac) and N-acetylglucosamine (GlucNac). Colorimetric substracts for lysozyme C are synthesised. The first would comprise an indol based compound linked to N-acetylglucosamine by a β 1-4 linkage. This compound would be colourless. Once the lysozyme cleaves the linkage, the indol compound is released turning the reaction mixture coloured (probably blue). Also there is an alternative compound where the N-acetylmuramic acid is linked to an indol based compound to give an alternative colorimetric compound. This can be a qualitative assay or quantitative by measuring the absorbance of the released indol based compound at its peak absorbance when excited at its preferred excitation wavelength. This can then be converted to absolute values for lysozyme activity in the extract.

(2) ANNEXIN TYROSINE PHOSPHORYLATION DETECTION

Reagents different from (1):

(4) PHOSPHATASE ACTIVITY IN TISSUE

Because the annexins are tyrosine dephosphorylated during disease progression/onset, there may be residual phosphatase activity still present in the tissue some time after the disease has started (assuming that in healthy tissue where there is no kinase activity, there is no phosphatase activity). It is assumed that this will eventually disappear with time (but on the other hand, it may be present throughout the disease). An additional test would be to test for phosphatase activity in the tissue. If phosphatase activity is present throughout the disease as a long term activity, then it gives us another disease marker. If however phosphatase activity disappears with time, then the absence of phosphatase activity and the absence of tyrosine phosphorylated annexins will indicate that the disease has been present for some time. The presence of phosphatase activity and the absence of tyrosine phosphorylated annexins will indicate a recent development of disease.

(A) Tissue biopsies will be extracted without sodium vanadate. 10-20 µl aliquots of extract will be placed on a blank area of a test strip (no immobilised antibodies in the defined area). This strip will bind all the proteins of the extract including the phosphatase. Aliquots of extract will be placed on the defined area of the test strip and left for 10-20 minutes. The immobilised phosphatase on the strip will be detected with the same AP substrate solution.

(B) A colorimetric assay which comprises placing aliquots of extract into a phosphatase substrate solution could also be used. The light absorbance would be measured at a certain wavelength. The substrate provides a detectable colour change and is a substrate for alkaline phosphatase.

(5) A COMMERCIAL TRIPLE TEST FOR LYSOZYME C ANNEXIN TYROSINE DEPHOSPHORYLATION AND PHOSPHATASE ACTIVITY

All three tests present on one strip. However, either the biopsies will have to be split between extraction with buffers with and without sodium

what, if any, mutations are present and where they are. A set of primers will be synthesised with the genetic base change(s) identified from the patients incorporated into the 5' end of the primers (one base change per primer). So a number of mutations could be envisaged in different patients causing lysozyme loss (although it should be noted that there maybe only one mutation type which causes disease in all patients). In the test kit, a set primer for these mutations (a mixture of all primers) will be used in conjunction with a standard primer with no mutation in it (from a region of the gene with no mutation identified in all patients) to amplify gene fragments by PCR. If any of the identified mutations, are present then a DNA fragment of known size will be amplified, this will allow us to determine where the mutation is in the gene. If no mutation is present then no DNA fragment will be amplified because of the 5' mismatch.

Alternatively, primers can be synthesised, which have the non-mutant sequences in the 5' end for all mutation regions of the gene. Using this primer set as a mixture, with the standard non-mutant primer. DNA fragments can be amplified using PCR on DNA from blood. If there is no mutation then a ladder of bands of known sizes corresponding to amplifications from each of the primers in the mixture to the standard primer will be obtained. If on the other hand, a known mutation is present, one of the bands in the ladder will be missing. Not only will we know that there is a mutation but also where it is in the gene.

Sizes of DNA bands will be evaluated using standard agarose gel electrophoresis. The kit will comprise the primer sets. Buffers to extract DNA from blood and the reagents needed to carry out the PCR.

A similar process can be envisaged for any other process of lysozyme loss if there is a genetic cause, eg illegitimate activation of a ubiquitin pathway of lysozyme C degradation.

These tests may also be valid for other conditions such as degeneration

Lysozyme C intradiscal replacement therapy restores the cellular/intracellular biochemical milieu that has been demonstrated to be altered in degenerate intervertebral discs derived from patients with low back pain and sciatica

B) Patient Selection criteria:

i) Back pain and/or sciatica unresponsive to conservative treatment:

Clinical symptoms:

- at least 6 months duration
- mild to moderate or appreciative functional disability

Diagnosis:

- supportive Magnetic resonance image scans showing disc degeneration and/or disc protrusion
- diagnostic test kit positive for Lysozyme C loss
- discography +ve for back pain +/- sciatic symptoms

ii) Sciatica unresponsive to conservative treatment:

Clinical symptoms:

- at least 6 months duration
- mild to moderate or appreciative functional disability

Diagnosis:

- supportive Magnetic resonance image scans showing disc protrusion
- diagnostic test kit positive for Lysozyme C loss
- discography +ve for sciatic symptoms

C) Therapy:

- using Image Intensifier (X-ray control)

Intradiscal injection therapy:

- using Image Intensifier (X-ray control)
- needle puncture of disc space under local anaesthetic
- determine whether disc pain +/- sciatic symptoms reproducible
- intradiscal injection of phosphatase inhibitor performed

3) STEROID THERAPY

A) Working Hypothesis:

Various forms of corticosteroids are known to induce the production of both annexin I and II. This may be used in combination with the intradiscal introduction of either Lysozyme C alone or with Lysozyme C and phosphatase inhibitors.

4) GROWTH FACTOR THERAPY

A) Working Hypothesis:

Annexin I and II are the major substrates for tyrosine activity associated with growth factors (e.g. epidermal growth factor (EGF) and platelet derived growth factor (PDGF-R)), and hormones (e.g. insulin). The introduction of these various factors will restore the phosphorylation status of these annexin proteins.

5) COMBINATION THERAPY

The following combination therapy may be possible:

(1) = Lysozyme C
(2) = Phosphatase inhibitor
(3) = Steroid
(4) = Growth factor

be understood that the Applicant claims protection in respect of any patentable feature or combination of features hereinbefore referred to and/or shown in the drawings whether or not particular emphasis has been placed thereon.

10. A method as claimed in any of claims 7 to 9, in which only the conjugated monoclonal antibody is used in western blotting and dot blotting.

11. A method as claimed in any of claims 7 to 10, in which the conjugated antibody and the unconjugated antibody are utilised in the enzyme linked immunosorbent assay.

12. A method as claimed in any preceding claim, in which the method comprises a colorimetric assay.

13. A method as claimed in claim 12, in which colorimetric substrates for lysozyme C are synthesised for use in the assay.

14. A method as claimed in claim 13, in which the substrate is an indol based compound linked to N-acetylglucosamine by a β 1-4 linkage.

15. A method as claimed in claim 13 or claim 14, in which the substrate is clear and the mixture of substrate and lysozyme C becomes coloured when lysozyme C cleaves the linkage.

16. A method as claimed in claim 13, in which the substrate is a compound where N-acetylmuramic acid is linked to an indol based compound.

17. A method as claimed in any of claims 14 to 16, in which the absorbance of the released indol based compound is measured at its peak absorbance when excited at its excitation wavelength.

18. Apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a support on which an unconjugated monoclonal antibody to lysozyme C is immobilised whereby an indication is given when the support comes into contact with lysozyme C.

used to treat joint disease.

29. A method of diagnosing low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said method comprising detecting the presence of tyrosine phosphorylation in tissue extracted from intervertebral discs or fluid extracted from large joints.

30. A method as claimed in claim 29, in which the amount of tyrosine phosphorylation is detected.

31. A method as claimed in claim 29 or claim 30, in which the method involves detecting the presence of annexin tyrosine phosphorylation.

32. A method as claimed in any of claims 29 to 31, in which the method comprises western blotting, dot blotting, or an enzyme linked immunosorbent assay.

33. A method as claimed in claim 32, in which two monoclonal antibodies are used in the tests.

34. A method as claimed in claim 33, in which the first antibody recognises tyrosine phosphate and is conjugated to an enzyme.

35. A method as claimed in claim 33 or claim 34, in which the second antibody recognises annexins and is unconjugated.

36. A method as claimed in any of claims 33 to 35, in which the first antibody is conjugated to alkaline phosphatase, horse radish peroxidase or biotin.

37. A method as claimed in any of claims 31 to 36, in which a colour change occurs when a tyrosine phosphorylated annexin is present.

47. Apparatus as claimed in any of claims 43 to 46, in which the apparatus is utilised in an enzyme linked immunosorbent assay using an enzyme conjugated monoclonal antibody to tyrosine phosphate.

48. Apparatus as claimed in claim 47, in which the enzyme is alkaline phosphatase, horse radish peroxidase or biotin.

49. Apparatus as claimed in claim 47 or claim 48, in which the substrate for the enzyme provides a colour change in the presence of tyrosine phosphorylated annexin.

50. Apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a support on which an unconjugated monoclonal antibody to annexin and an unconjugated antibody to lysozyme C are immobilised whereby an indication is given when the support comes into contact with tyrosine phosphorylated annexin and lysozyme C.

51. Apparatus as claimed in claim 50, in which the two antibodies are immobilised on separate areas of the support.

52. Apparatus as claimed in claim 50 or claim 51, in which the support comprises a test strip or a nitro-cellulose/ nylon sandwich membrane.

53. Apparatus as claimed in claim 52, in which the test strip comprises opaque polystyrene or another support matrix which is able to bind antibodies.

54. Apparatus as claimed in any of claims 50 to 53, in which the support is pre-blocked with blocking agents when the unconjugated antibodies have been immobilised thereon.

55. Apparatus as claimed in any of claims 50 to 54, in which the apparatus is

64. A method as claimed in claim 63, in which a substrate for alkaline phosphatase provides the colour change in the presence of immobilised phosphatase.

65. A method as claimed in any of claims 58 to 64, in which the method comprises a colorimetric assay.

66. A method as claimed in claim 65, in which the assay comprises placing aliquots of extract into a phosphatase substrate solution and measuring the light absorbance at a certain wavelength.

67. A method as claimed in claim 66, in which the substrate is a substrate for alkaline phosphatase which provides a colour change in the presence of phosphatase.

68. A method of treatment of low back pain and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of phosphatase inhibitor.

69. A method as claimed in claim 68, in which the phosphatase inhibitor is administered by injection into a vertebral disc or joint.

70. A method as claimed in claim 68 or claim 69, in which the inhibitor is administered in combination with lysozyme C.

71. A method as claimed in any of claims 68 to 70, in which the method is also used to treat joint disease.

72. A method of treatment of low back paid and sciatica, comprising the administration, to a body in need of such treatment, of an effective dose of tyrosine phosphorylated annexin.

73. A method as claimed in claim 72, in which the method may be used to

82. Apparatus for use in the diagnosis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases, or a predisposition thereto, said apparatus comprising a set of primers with non mutant sequences in the 5' end for all mutation regions of the lysozyme C gene for use in PCR amplification of DNA fragments.

83. Apparatus as claimed in claim 82, in which the apparatus is utilised for detecting the presence of mutations in the lysozyme C gene.

84. Use of lysozyme C as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

85. A method as claimed in claim 84, in which the lysozyme C is injected into the disc or joint.

86. A method as claimed in claim 85, in which the lysozyme C is injected in combination with a growth factor.

87. The use of lysozyme C in the manufacture of medicament for the treatment of back pain and sciatica.

88. Use of a phosphatase inhibitor as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

89. The use of a phosphatase inhibitor in the manufacture of a medicament for the treatment of back pain and sciatica.

90. Use of corticosteroids as a therapeutic agent in the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

diluent or carrier.

100. A composition as claimed in claim 99, in which the composition is used for the treatment of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint disease, or a predisposition thereto.

101. A method of diagnosing low back pain and sciatica associated with degenerative disc disease and degenerative joint disease, or a predisposition thereto, said method comprising introducing a lysozyme C substrate analogue in tissue of intervertebral discs or large joints and analysing the magnetic resonance of the substrate with nuclear magnetic resonance techniques.

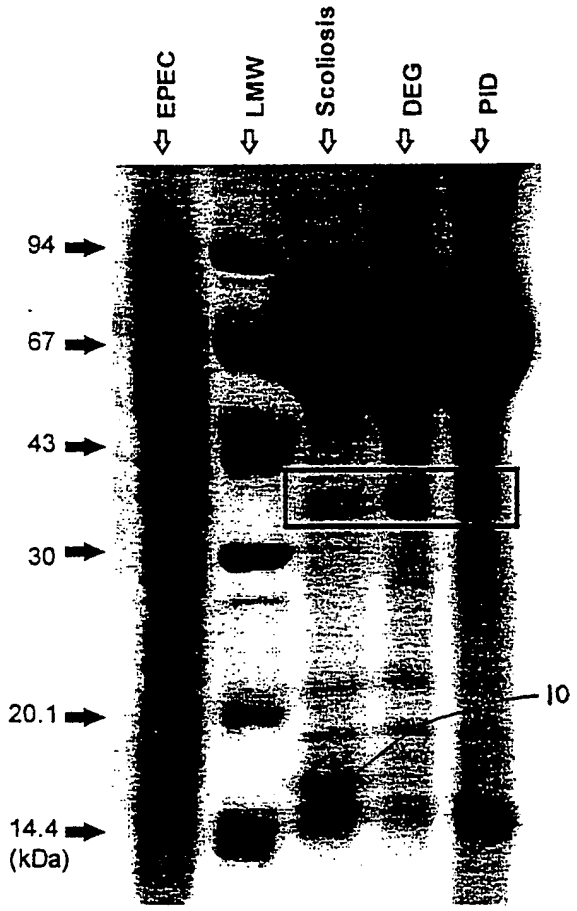
102. A method as claimed in claim 101, in which the analogue is introduced by injection.

103. A method as claimed in claim 101 or 102, in which the analogue comprises a substrate homologue with a beta 1-4 linkage.

104. A method as claimed in any of claims 101 to 103 in which the method involves analysis of the degree of change in the magnetic resonance properties of the homologue, which may provide for determination of the degree of disease progression.

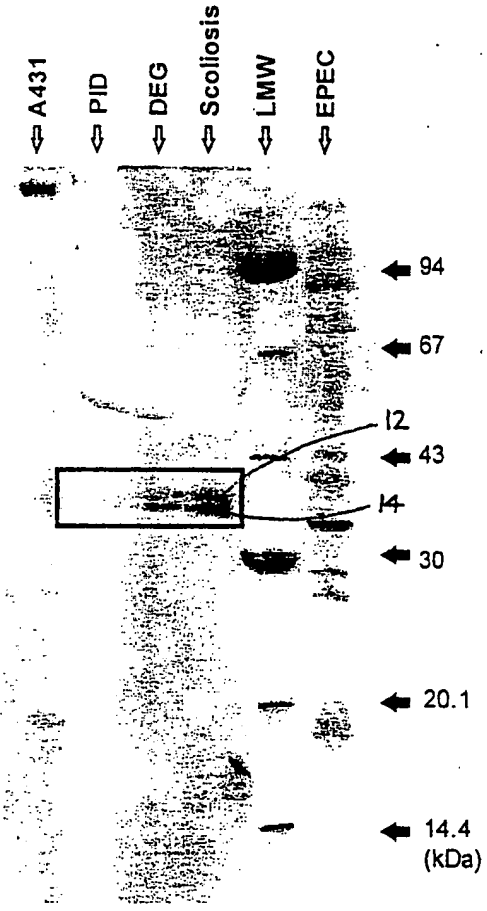
105. The use of lysozyme C substrate analogue in the analysis of low back pain and sciatica associated with degenerative intervertebral disc disease and degenerative joint diseases.

106. The use of lysozyme C substrate analogue as claimed in claim 105, in which the magnetic resonance characteristics of analogue introduced into tissue of intervertebral discs and joints are analysed using nuclear magnetic resonance techniques.


EPEC
LMW
Scoliosis
DEG
PID
94
67
43
30
20.1
14.4
(kDa)
10

(a)

FIG. 1


A431
PID
DEG
Scoliosis
LMW
EPEC
94
67
43
30
20.1
14.4
(kDa)
12
14

(b)

FIG. 2